Letter to the Editor

Toluene downregulates filaggrin expression via the extracellular signal-regulated kinase and signal transducer and activator of transcription–dependent pathways

To the Editor:

Volatile organic compounds (VOCs) can be an important environmental risk factor in the development and/or aggravation of atopic dermatitis (AD). A previous study reported that xylene and formaldehyde intensified allergic inflammation in an experimental mouse model.[1] In a prospective study to evaluate the clinical effects of air pollution including VOCs on skin symptoms in children with AD,[2] concentrations of total VOCs were higher on days when the patients had symptoms of AD than on days without symptoms. A significant increase in transepidermal water loss after exposure to VOCs was also reported.[3] Although these accumulated data show an association between exposure to VOCs and aggravation of AD, the molecular effects of VOCs on the aggravation of AD are still unclear.

Filaggrin is a key regulator in epidermal barrier function. The pathogenesis of AD may be proposed as an epidermal barrier defect that leads to diminished epidermal defense mechanisms.[3] In a cohort study, filaggrin mutations were associated with the predilection sites of AD that are exposed areas of the body such as hands and cheeks, whereas there were no predilection sites in persons with the wild-type gene.[4] These sites are typically exposed to environmental factors including irritants, air pollution, and chemicals. This suggests that persons with mutations in genes encoding proteins of the skin barrier are susceptible to AD when exposed to environmental triggers. On the basis of these reports, we investigated the effects of toluene, a typical VOC that usually occurs in indoor air from the use of common household products (paints, paint thinners, adhesives, synthetic fragrances, and nail polish) and cigarette smoke, on filaggrin-mediated epidermal barrier dysfunction.

After toluene exposure, the expression of filaggrin mRNA and protein decreased (Fig 1, *A* and *B*). Furthermore, to determine whether toluene affects the expression of other keratinocyte differentiation-associated markers (eg, involucrin, loricrin, keratin 10, and keratin 5), we measured the mRNA levels of each of these molecules after toluene treatment. The present study demonstrated that toluene did not significantly affect the expression of involucrin, loricrin, keratin 10, and keratin 5 (data not shown). Fluorescence microscopy analysis also showed that filaggrin levels were lower in toluene-treated human skin equivalent models than in untreated human skin equivalent models (Fig 1, *C*). In addition, we evaluated the filaggrin breakdown products such as natural moisturizing factor (NMF) associated with transepidermal water loss. Pyrrolidone carboxylic acid and urocanic acid are known as the main components of NMF. As a result, levels of NMF components (pyrrolidone carboxylic acid/urocanic acid) were decreased by toluene treatment (see Fig E1 in this article's Online Repository at www.jacionline.org).

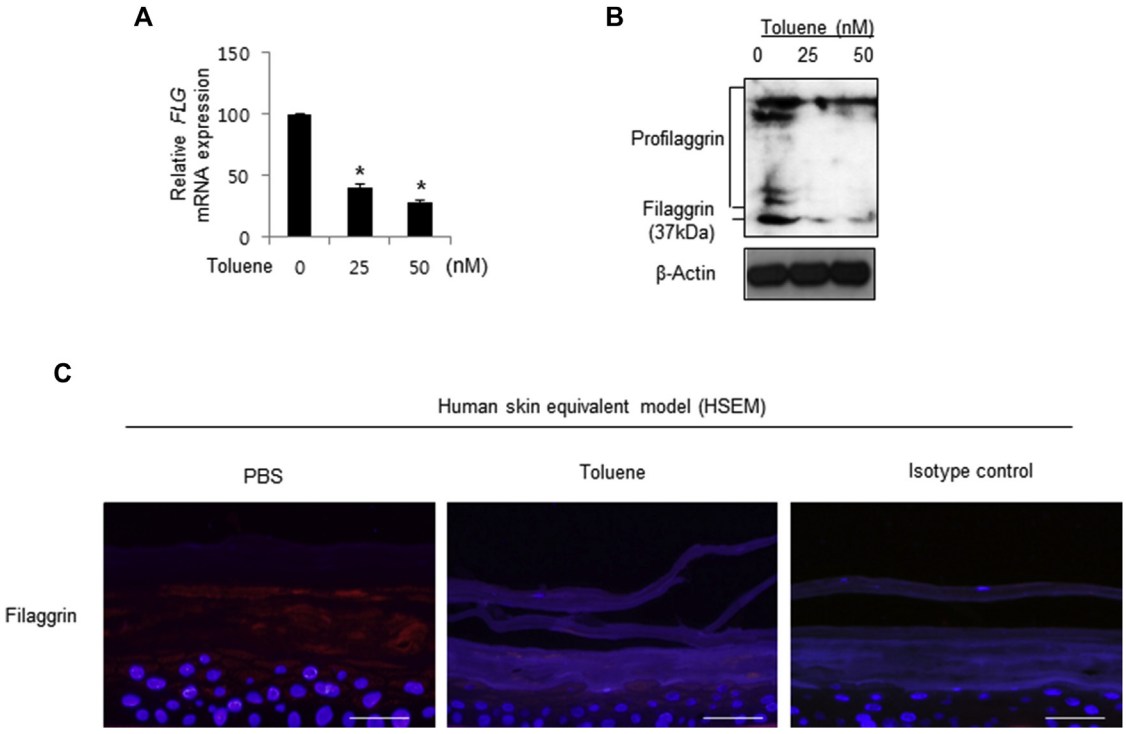

FIG 1. Toluene inhibits filaggrin expression in keratinocytes in a dose-dependent manner. Filaggrin expression was decreased by toluene exposure at the mRNA **(A)** and protein level **(B)** in keratinocytes. Downregulated filaggrin protein expression was detected by immunofluorescence staining in HSEMs **(C)**. *HSEM,* Human skin equivalent model. Columns and error bars represent means ± SEMs from 3 independent experiments. **P* < .05. Bar = 50 μm.

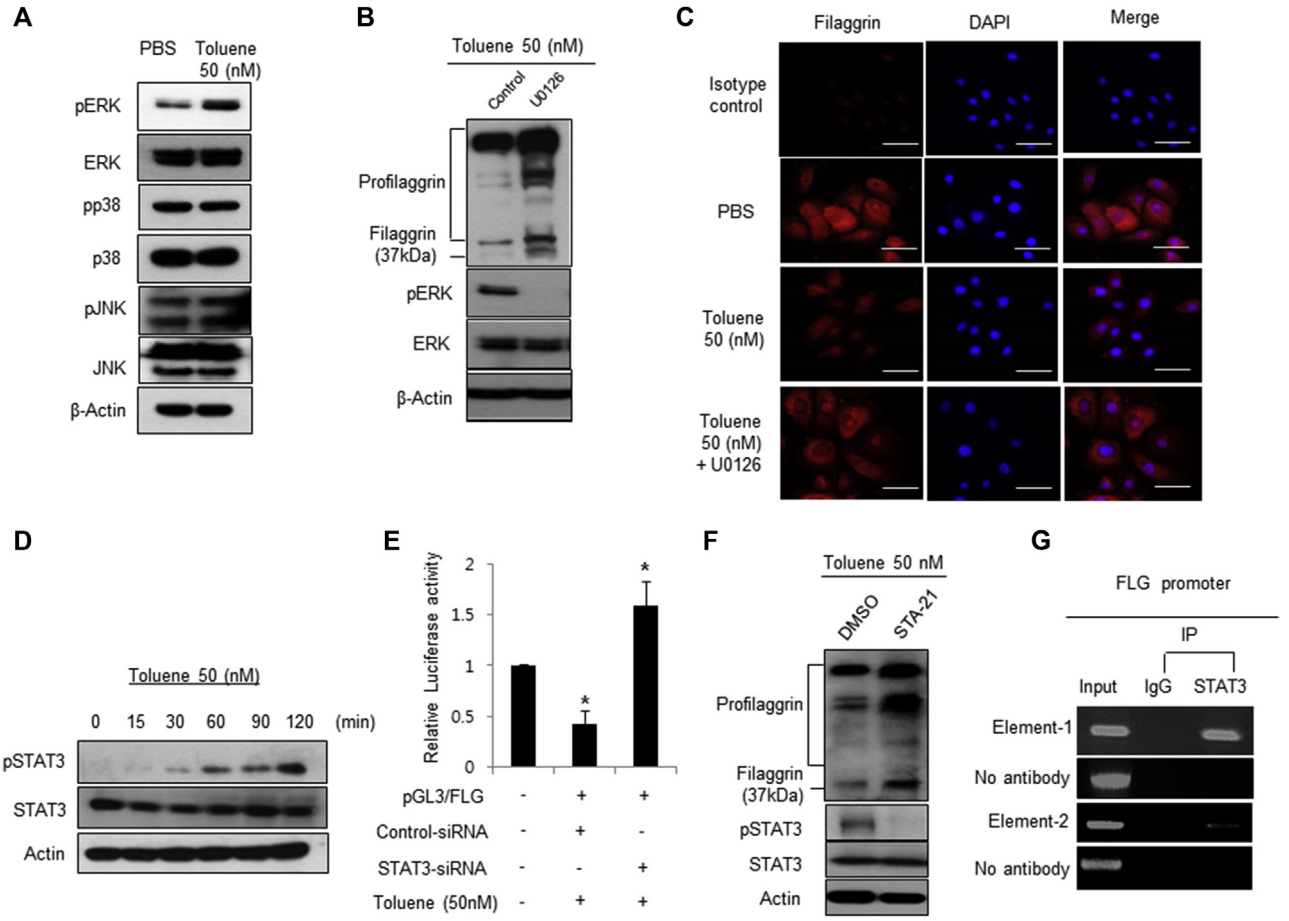

FIG 2. Toluene induces the downregulation of filaggrin expression by ERK/STAT-3– dependent mechanism. Toluene increased the ERK phosphorylation in keratinocytes **(A)**. Inhibition of ERK restored the toluene-mediated downregulation of filaggrin expression shown by Western blot **(B)** and immunofluorescence assay **(C)**. Phosphorylated STAT-3 protein expression was increased after toluene exposure **(D)**. Downregulated filaggrin expression was upregulated by STAT3 siRNA at the promoter activity **(E)**, protein level **(F)**, and after toluene exposure. Results of chromatin immunoprecipitation assay showed that the STAT-3 binds directly to the putative STAT-3 binding sequence of the filaggrin promoter **(G)**. *DAPI*, 4′-6-Diamidino-2-phenylindole, dihydrochloride; *DMSO*, dimethyl sulfoxide. Columns and error bars represent means ± SEMs from 3 independent experiments. $*P < .05$. Bar = 100 μm.

The involvement of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase in cells under stress due to exposure to benzene, toluene, and formaldehyde has been reported.[5] To understand the regulatory mechanisms of toluene-induced filaggrin downregulation, our study investigated whether the mitogen-activated protein kinase signaling pathway is induced in keratinocytes after toluene exposure. Toluene increased the activation of ERK phosphorylation in keratinocytes. However, it did not induce noticeable phosphorylation of p38 and JNK (Fig 2, *A*). Pretreatment with the ERK inhibitor U0126 markedly restored filaggrin expression suppressed by toluene exposure (Fig 2, *B* and *C*). But suppression of p38 with SB239063 or JNK with SP600125 did not alter filaggrin expression (see Fig E2 in this article's Online Repository at www.jacionline.org). To further establish the role of ERK1/2 activation, we used siRNA-mediated incapacitating approach and assessed the filaggrin expression. Treatment with siRNA effectively attenuated ERK1/2 and resulted in restoration of filaggrin expression (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). These results suggest that the activation of ERK is required for toluene-induced filaggrin downregulation.

It is known that ERK can be involved in the regulation of signal transducer and activator of transcription 3 (STAT3) expression in cells. A recent study has shown that STATs may be important mediators of environmental factor, toluene-induced inflammatory disease.[6] Previously, we reported that thymic stromal lymphopoietin suppression of filaggrin expression is STAT3 dependent.[7] Therefore, we assumed that STAT3 may regulate toluene-induced filaggrin downregulation in keratinocytes. After toluene treatment, STAT3 protein levels peaked after 2 hours (Fig 2, *D*). After treatment with 50 nM of toluene, filaggrin promoter activity was higher in STAT3 siRNA-transfected keratinocytes than in nontargeting siRNA-transfected keratinocytes (Fig 2, *E*). To confirm the STAT3 dependency of filaggrin reduction, we analyzed the effect of STAT3 on filaggrin expression by STAT3-specific inhibitors (STA-21). Compared with control, in the keratinocytes treated with STA-21, filaggrin expression increased at the protein levels (Fig 2, *F*). Moreover, treatment with siRNA effectively attenuated STAT3 and resulted in